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GLUTATHIONE MEDIATED RESISTANCE AGAINST *FUSARIUM OXYSPORUM* INDUCED OXIDATIVE STRESS IN WHEAT (*TRITICUM AESTIVUM*) SEEDLINGS

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ABSTRACT: In the present investigation, it was found that priming of wheat seedlings with 50 µM concentration of aluminium (Al) induces over-expression of total glutathione. After 48 hrs the abundance in total glutathione in Al pre-treated seedlings increased by 60% as compared to untreated control seedlings. The abundance in total glutathione was proportional to the increase in the reduced form of glutathione. The abundance in glutathione content was found to be linked with the enhancement of γ-ECS activity. The enzymatic activity of γ-ECS was maximum after 16 hrs of Al application. Whereas, wheat seedlings treated with 1mM concentration of BSO for 48 hrs showed 30% decline in total glutathione content coupled with decline in γ-ECS activity. To assess the role of over-expressed glutathione during plant-pathogen interaction Al primed seedlings were infected with *Fusarium oxysporum* spores. Studies on growth parameter, DSI and ROS activity established over-expressed glutathione as a key player in mitigating *Fusarium* induced oxidative stress on wheat seedlings. BSO treated seedlings infected with *Fusarium* were highly susceptible to the pathogen. Over-expressed glutathione maintains the cellular redox balance by regulating ROS generation. Controlled accumulation of ROS restricts pathogen entry thereby, making the host resistant to pathogen. Thus, pre-treatment with Al up-regulates glutathione synthesis which protects wheat seedling against *Fusarium*.

Keywords: *Fusarium oxysporum*, *Triticum aestivum,* aluminium, glutathione, disease severity index, reactive oxygen species

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1.INTRODUCTION

The tri-peptide glutathione is widely distributed in prokaryotes and eukaryotes [1]. It is the most important non-enzymatic sulfur containing antioxidant in plants and is a key regulator in plant defense against biotic and abiotic stress. Glutathione is known for playing critical role in the detoxification of the reactive oxygen species (ROS) either directly or through the ascorbate-glutathione pathway. Glutathione participates in redox signaling, modulation of gene expression [2] and regulation of enzymatic activities [3]. Glutathione is also associated with detoxification of herbicides, xenobiotics, heavy metals [4] involved in the biosynthesis of sulfur containing plant defense compounds and is involved in the process of glutathionylation [5]. Glutathione is synthesized in two ATP driven steps triggered by enzymes. In the first step cysteine is linked to glutamate to form γ-glutamylcysteine and is catalyzed by the enzyme γ-glutamylcysteine synthetase. In the second step glycine is linked to γ-glutamylcysteine by the enzyme glutathione synthetase to form glutathione. Glutathione is recycled through the oxidation/reduction process of its reduced (GSH) and oxidized (GSSG) forms. Glutathione is present in non-stressed plants mainly in its reduced form, whereas during oxidative stress high amounts of oxidized glutathione are formed. High amount of oxidized glutathione relates to reduced growth and cell death [6]. Glutathione concentration increases in plants following pathogen infection [7]. γ-glutamylcysteine synthetase is the rate limiting step for the overall glutathione biosynthesis [6]. Plants with deficient γ-glutamylcysteine synthetase mutations show reduced levels of glutathione and exhibit highly susceptible phenotype to pathogens [1]. Glutathione affects the reactive oxygen species (ROS) thereby participating in the hypersensitive reaction initiated as a resistant response by plants perceiving pathogen attack [8]. The importance of glutathione in defense against a variety of pathogens as well as induction of defense genes has been extensively studied. In barley-powdery mildew interaction, a transient oxidation of glutathione correlates with H_2O_2 accumulation in the mesophyll cells [9]. NPR1, a protein regulator of pathogen resistance, is regulated by the redox state of cysteine which in turn regulates glutathione redox state [10]. Changes in the redox state of cysteine residues of proteins are strongly affected by that of glutathione. Therefore the changes in the cellular redox state of glutathione following plant-pathogen interactions has been postulated as a trigger for the cellular signaling required for appropriate defense responses to pathogens [11]. Glutathione acts in thiol-disulfide exchange reactions and links expression of gene regulation to the

redox state of cells [12]. Thiol-disulfide status appears to be important in disease resistance signaling. Exogenous application of glutathione has been reported to activate expression of a number of defense and stress related genes and proteins as well as PR proteins [13]. Reduction in the overall cellular glutathione content in plants has a profound effect on plant defense against pathogens [14]. The aim of the present study was to identify the origin of glutathione up-regulation in Al pre-treated seedlings and to investigate the putative role of over-expressed glutathione in pathogen resistance in wheat seedlings

2. MATERIALS AND METHODS

Plant materials and growth conditions: Hundred number of healthy wheat seeds selected for germination were surface sterilized with 0.1% mercuric chloride, followed by washing thrice with sterile distilled water. The surface sterilized seeds were placed for germination on filter paper soaked with deionised water and incubated at 26°C in the dark for 48 hours. Germinated seedlings with uniform root growth were transferred to sterile glass tubes containing hydroponic solution and kept in a growth chamber at 26°C and 80% relative humidity during 14 h light period and at 22°C and 70% relative humidity during the 10 h dark period for seven days.

Chemical treatments and pathogen inoculation: Seven days old germinated wheat seedlings were transferred to sterile glass tubes each containing 10ml hydroponic solution. One set of tubes containing germinated seedlings were each incubated with 1ml of 50 μM aluminium (Al) solution and maintained at room temperature (RT) for 48 hours. Another set of tubes with germinated seedlings were incubated with 1ml of 1mM BSO (Dl-buthionine-SR-sulfoximine) suspension for 48 hrs at RT. Another set of seedlings were similarly incubated with distilled water and maintained under similar conditions. Seedlings pre-incubated with 50 μM Al, 1mM BSO and distilled water after 48 hours were transferred to sterile glass tubes and inoculated with freshly prepared 4 days old *Fusarium oxysporum* spores (1×10^6 dilution) and the seedlings were allowed to grow for another 7 days.

Assay of glutathione: Leaf tissue (0.1gm) was homogenized in pre-chilled mortar and pestle with 3% (v/v) trichloro-acetic acid (TCA) followed by centrifugation at 10,000 rpm for 10 mins at 4°C. The supernatant was used for estimation of total glutathione using Ellman's reagent (DTNB i.e., 5,5′-dithiobis nitro benzoic acid). The absorbance was recorded at 412 nm in a UV-VIS spectrophotometer (Systronics) [15]. The concentration of oxidised glutathione was estimated in the same reaction mix after addition of 4-vinylpyridine. The concentrations of total and oxidised glutathione were calculated from standard curve obtained from authentic GSH and GSSG (Sigma, USA). The redox state of glutathione was given by ratio between reduced glutathione (GSH)/total glutathione (GSH+GSSG).

Hydrogen peroxide (H_2O_2): H_2O_2 concentration was estimated following the method of [16]. 0.2g fresh leaf and root tissue was separately homogenized in a pre-chilled mortar and pestle with 0.1%

TCA. The homogenate was centrifuged at 12000g (rpm) for 15mins at 4ºC. The supernatant was collected and used to estimate H_2O_2 content. The reaction mixture consisted of 10mM K-phosphate buffer (pH 7.0) and 1M KI. 0.5ml of the plant extract was added to 0.5ml 10mM K-phosphate buffer (pH 7.0) and 1ml of 1M KI and incubated at RT for 20 mins. The readings from the sample plant extracts were recorded against a blank containing 0.5ml of 0.1% TCA along with the reaction mixture without plant extract. The test tubes at all times were placed inside light impenetrable box which was place on ice. The absorbency of the mixture was read at 390 nm. The concentration of H_2O_2 in the sample extract was determined from the standard curve by regression analysis prepared from commercially available 30% H_2O_2 solution.

Enzyme assay

γ-glutamylcysteine synthetase activity:γ-glutamylcysteine synthetase (γ-ECS, EC 6.3.2.2) was extracted and measured following the method described by [15]. 0.1 gm of leaf tissue was homogenized with 0.1M hydrochloric acid (HCl) in a pre-chilled mortar and pestle and the homogenate was centrifuged at 20,000×g for 10 mins at 2°C. The supernatant was used for assay of enzyme activity. To 500 µl of enzyme extract 1ml of assay mixture consisting of 50mM Tris-HCl (pH 7.6) containing 0.25mM glutamate, 10mM ATP, 1mM dithioerythritol and 2mM cysteine was added and incubated at 25ºC for 1 hr to initiate the reaction process. Then 1.2ml of phosphorous agent containing 3mM H_2SO_4, distilled water, 2.5% ammonium molybdate and 10% vitamin C was added and mixed thoroughly. The mixture was incubated at 45ºC for 25 mins. The mixture was cooled to RT. The absorbance was recorded at 660 nm in a UV-VIS spectrophotometer. One unit of γ-ECS activity was defined as 1µmol Cysteine dependently generated Po^{3-}_4 per minute. A molar coefficient of 5.6 mM-1cm-1 was used for calculating enzyme activity. The specific enzyme activity was expressed as unit/mg protein.

Peroxidase (POX): POX activity was measure following the method described by [17]. 0.1g fresh leaf and root tissue was homogenized in a pre-chilled mortar and pestle with 0.1M potassium phosphate buffer (pH 6.6). The homogenate was centrifuged at 18,000g for 15mins at 4ºC. In a clean and dry test tube 3ml of 0.05M pyrogallol solution was taken to which 0.1ml of enzyme extract was added and incubated for 10 mins on ice. Next 0.5ml of 0.5% H_2O_2 solution was added and mixed well. The reaction mixture was incubated for 5 mins. The absorbance of the reaction mixture was read at 420 nm for every 30 secs up to 3 mins in a UV-VIS spectrophotometer against a blank containing only phosphate buffer and no plant enzyme extract. POX enzyme activity was calculated using molar extinction co-efficient $2.47mM^{-1}cm^{-1}$ and expressed as units/mg protein

Polyphenol oxidase (PPO): PPO activity was measure following the method described by [18]. Fresh leaf tissue and root tissue (0.1g) was homogenized in a pre-chilled mortar and pestle with 10mM sodium phosphate buffer pH 6.5. The homogenate was centrifuged at 12,000g for 20 mins at 4ºC. The reaction was initiated by adding 2.5ml of Na-phosphate buffer to 0.2ml of plant enzyme

extract taken in clean and dry test tubes. The test tubes were incubated for 20mins at RT. 0.3ml of catechol solution was added to the same test tubes and mixed well. The absorbance of the reaction mixture was read at 495nm for every 30 secs up to 5 mins. Reaction mixture containing phosphate buffer without plant enzyme extract served as control. The activity of PPO was calculated using molar extinction co-efficient for oxidised catechol $3.6\times10^{4}M^{-1}cm^{-1}$.

Phenylalanine ammonia lyase (PAL): PAL activity was estimated following the method described by [19]. 0.1g fresh leaf and root tissue was separately homogenized in a pre-chilled mortar and pestle with 25mM borate buffer (pH 8.8). The homogenate was centrifuged at 8000g for 30 mins at 4ºC. The supernatant collected was used to estimate PAL activity. In clean and dry test tube 1ml of plant enzyme extract was added to 0.5ml of 50mM L-phenylalanine and 0.4ml of 25mM borate buffer. The tubes were incubated for 2hrs. at 40ºC in a water bath. The reaction was stopped by removing the tubes from the water bath and adding 0.06ml of 5N HCl. The absorbance was read at 290 nm against the same volume of reaction mixture without L-phenylalanine, which served as blank. Molar extinction co-efficient of trans cinnamic acid 10,900L/molcm was used to calculate PAL activity and expressed as nmoles of trans cinnamic acid per minute per mg protein

Glutathione reductase (GR): GR activity was measured following the method described by [20]. 0.5g fresh leaf and root tissue was homogenized with ice-cold K-phosphate buffer (pH 7.5) and the homogenate was centrifuged at 15,000g for 30 mins at 4ºC and the supernatant collected was used as the enzyme source. The 2ml reaction mixture consisted of 1.65 ml of 50mM K-phosphate buffer (pH 7.0), 100µl $MgCl_2$ (3.5mM), 50 µl of oxidised glutathione (GSSG) and 100 µl of the sample supernatant. The reaction mixture was started by adding 100 µl of NADPH and change in absorbance was read at 340 nm for 5 mins. The blank consisted of the components of the reaction mixture except for the plant enzyme extract, which was replaced by an equal volume of the assay buffer. An additional blank without oxidised glutathione was used to account for the presence in the plant extract of other enzyme capable of oxidizing NADPH. The activity of was reported as nmoles of NADPH oxidised/mg protein/minute using molar extinction co-efficient 6.22 mmol/L/cm. One GR unit was defined as the amount of enzyme that oxidizes 1µmol min^{-1} NADPH under the above assay conditions.

Growth parameters: Seedlings were removed from hydroponic solutions and rinsed thoroughly with sterile distilled water. The seedlings were patted dry using tissue paper. The seedling length and fresh weight of the seedlings were recorded immediately. The shoots of each seedling was removed from their respective roots. Root length and shoot length of the seedlings was measured. To determine dry weight the whole seedlings were carefully transferred to paper envelopes and dried at 70˚C for 12 hours in a hot air oven and dry weight of the seedlings were measured and recorded.

Disease severity index (DSI): Disease severity index was calculated according to [21]. The extent of infection was indicated by the condition of the root system of the plant. Healthy and infected seedlings were divided into four groups: 0, symptomless healthy seedlings; 1, slightly infected seedlings with dark brown roots; 2, heavily infected roots with weak and stunted growth; 3, dead and fallen seedlings. The disease severity index was calculated using the formula:

$$DSI = [0(H^n) + 1(S^n) + 2(HI^n) + 3D^n)] \div total \ \ no.of \ \ plants \ \ examined$$

Where, (H^n) = No. of healthy plants; (S^n) = No. of slightly infected plants; (HI^n) = Heavily infected plants; (D^n) = No. of dead plants

Assay of reactive oxygen species (ROS): ROS production was measured as described by [22] with some modifications by monitoring the reduction of 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide-inner salt (XTT) in presence of ROS. Leaves were homogenized in 50mM K-phosphate buffer (pH 7.8) and centrifuged at 5000rpm for 10mins. The reaction mixture contained 1ml of K-phosphate buffer (ph 7.8), 500µl of 0.5mM XTT and 500µl of extract. The reaction of XTT was determined at 470nm for 3mins. Corrections were made for the absorbance of chlorophyll. ROS production was calculated using extinction coefficient of 2.16×10^4 $M^{-1}cm^{-1}$. The estimated ROS generated was expressed as µM/gm fresh weight.

Histological study: Seedlings were removed from test solutions and 0.1 cm from leaf tips were excised asceptically with sterile blade. The leaf sections were decolourised with acetic acid: ethanol: water (2:2:1) solution at 25°C. The sections were next stained with lactophenol cotton blue with two changes following the method by [23] to view fungal structures inside the tissue using light microscope.

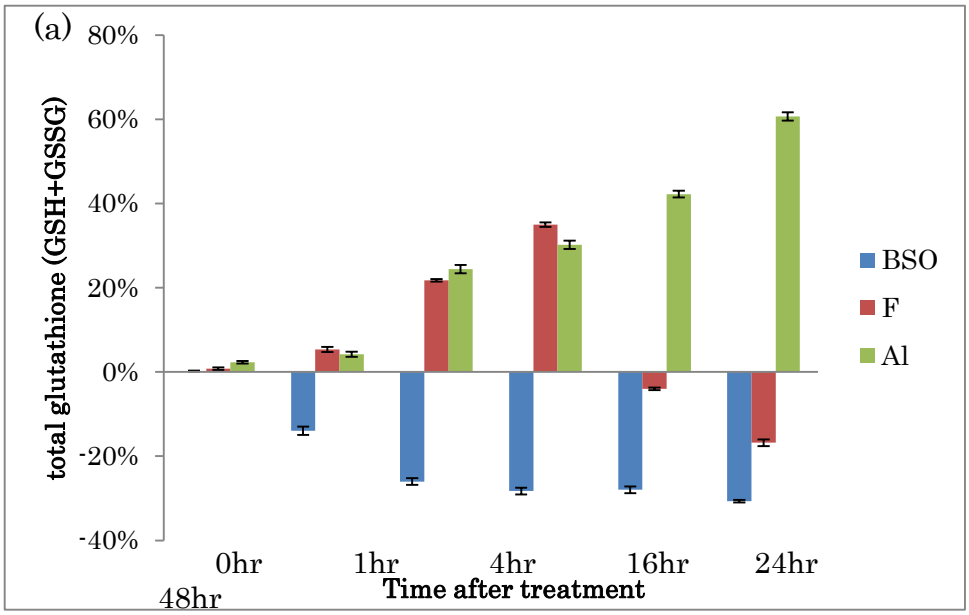
Statistical analysis: Student's t test was used to analyse statistically significant differences between different treatments. Significant differences were indicated for $p\leq0.05$ and $p\leq0.01$.

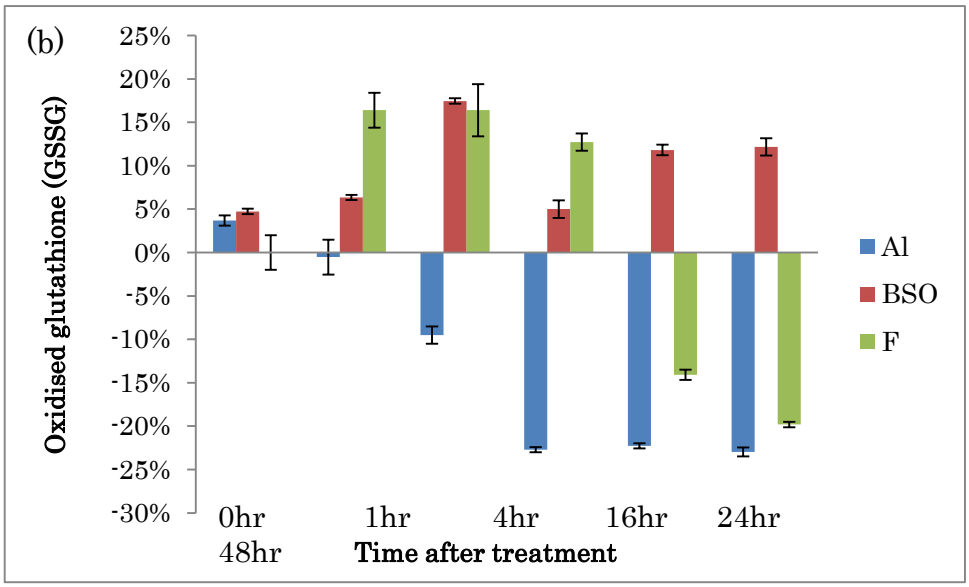
3. RESULTS AND DISCUSSION

The level of total glutathione was observed to increase steadily in case of Al primed seedlings (Fig 1a). The increase became apparent as early as four hours after application of Al. After 48 hours the abundance in total glutathione was increased by 60% as compared to control seedlings. The increase in total glutathione abundance was due to a proportional increase in the reduced form of the glutathione (GSH) as compared to the oxidized form (GSSG) (Fig 1b& 1c). The ratio of GSH/(GSH+GSSG) changed significantly. To determine if the increase in total glutathione abundance is due to increased synthesis, the activity of γ-ECS in wheat seedlings was determined. The enzymatic activity of γ-ECS increased steadily in Al pre-treated wheat tissues starting from four hours after Al application and was significantly different from untreated control seedlings (Fig 3). The increase in γ-ECS activity reached maximum at sixteen hours after Al application. Although the γ-ECS activity was increased after 24 hr and 48 hr, not much difference could be observed.

BSO treated seedlings showed a steady decline in the level of total glutathione (Fig 1a). The

decline became evident after 1 hour of application of BSO. After 48 hours the total glutathione content was 30% less as compared to untreated control seedlings. The decrease in glutathione content was due to proportional decline in the reduced form of glutathione (GSH) (Fig 1c). The ratio of GSH/GSSG changed significantly (Fig 2). The activity of γ-ECS declined rapidly in after one hour of application of BSO (Fig 3).





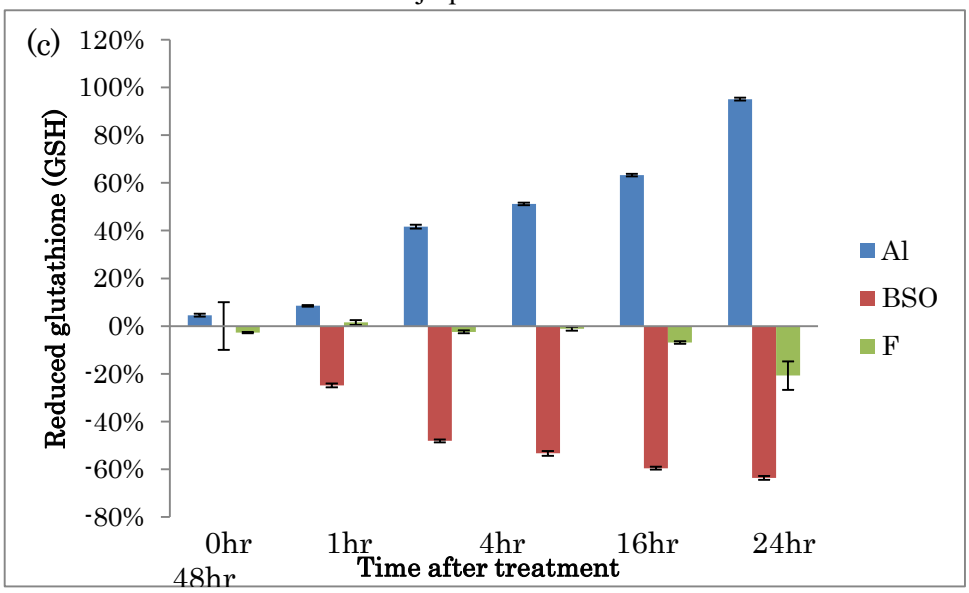


Figure 1. Percentage change in the (a) total glutathione content (GSH+GSSG), (b) oxidised glutathione content (GSSG) & (c) reduced glutathione content (GSH) in wheat seedlings at different time points. Al-represents data from 50 µM Al pre-treated seedlings. BSO-represents data from 1mM BSO pre-treated seedlings. F-represents data from *Fusarium* infected seedlings. Error bars indicate standard error.

The abundance of total glutathione increased in case of control seedlings inoculated with *Fusarium* spores (Fig 1). The increase was apparent after 1 hour of inoculation. At sixteen hours the abundance of total glutathione content was 35% higher in case of infected seedlings as compared to untreated control seedlings. The increase in total glutathione content was due to a proportional increase in the oxidized form (GSSG) of glutathione (Fig 1a). However, at 24 and 48hpi the abundance of total glutathione declined as compared to untreated control seedlings. The enzymatic activity of γ-ECS did not change significantly in case of infected seedlings (Fig 3).

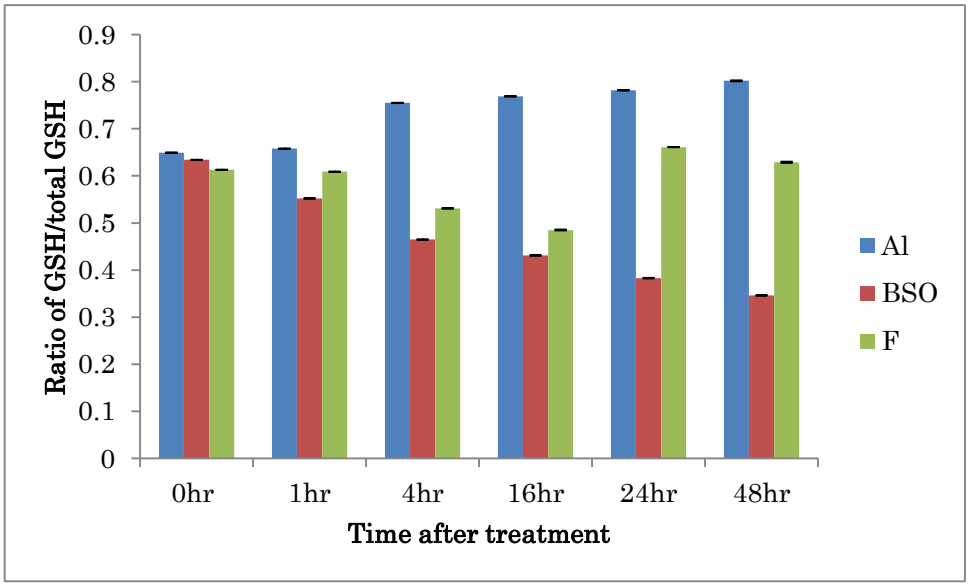


Figure 2. Ratio of reduced glutathione (GSH)/total glutathione in wheat seedlings at different time points. Al-represents data from 50 µM Al pre-treated seedlings. BSO-represents data from 1mM

BSO pre-treated seedlings. F-represents data from *Fusarium* infected seedlings. Error bars indicate standard error.

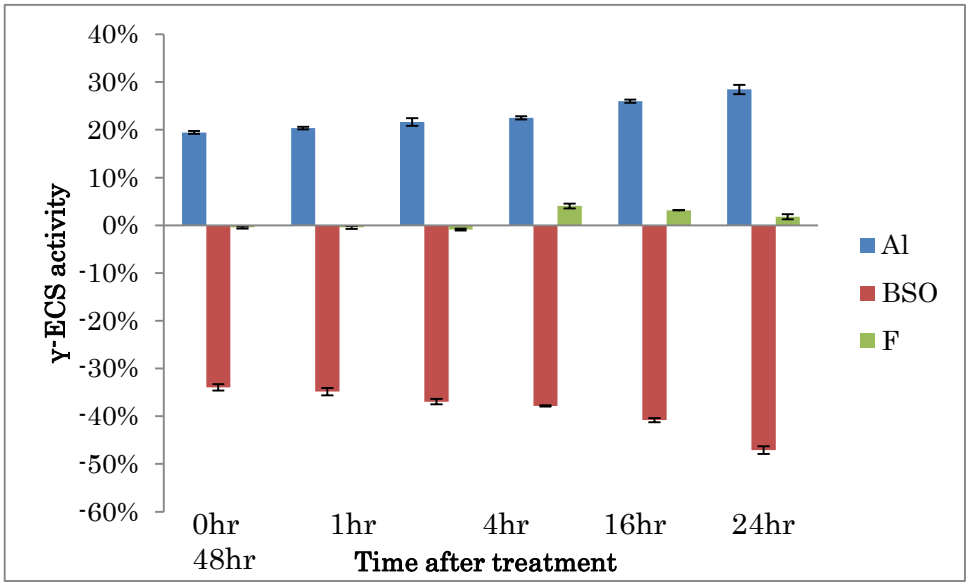


Figure 3. Percentage change in enzymatic activity of γ-ECS in wheat seedlings at different time points. Al-represents data from 50 µM Al pre-treated seedlings. BSO-represents data from 1mM BSO pre-treated seedlings. F-represents data from *Fusarium* infected seedlings. Error bars indicate standard error.

At 7 dpi Al pre-treated wheat seedlings infected with *Fusarium* showed no differences ($p<0.05$) in plant growth with respect to uninfected control plants. *Fusarium* infected seedlings showed reduction in all growth parameters. At 7 dpi BSO treated seedlings inoculated with fungal spores showed severe reduction in growth and biomass content as compared to control seedlings infected with *Fusarium* (Table 1).

Table 1. Changes in growth parameters and mean DSI due to inoculation with *Fusarium oxysporum* on wheat seedlings. C- data from untreated control seedlings, F-data from untreated control seedlings infected with *Fusarium*. Al+F-data from Al pre-treated seedlings infected with *Fusarium*. BSO+F-data from BSO pre-treated seedlings infected with *Fusarium*. Data represent mean value ± SE from three replicates, n=10. * and ** represent significant and highly significant differences as compared to untreated control at $p\leq0.05$ and $p\leq0.01$ respectively.

Sample	Root length	Shoot length	Seedling length	Fresh weight	Dry weight	Mean DSI
C	8.9±0.06	11.1±0.1	14.9.1±0.1	1.97±0.02	0.63±0.005	-
F	4.7±0.06*	6.7±0.1*	9.6±0.1*	0.85±0.01*	0.31±0.01**	58.76±0.2*
Al+F	8.5±0.05	10.3±0.1	15.5±0.1	1.92±0.02	0.56±0.008	12.88±0.1*
BSO+F	4.4±0.08*	6.6±0.06**	9.7±0.1**	0.72±0.01*	0.23±0.003**	77.1±0.1**

Resistance induced in wheat seedlings by 50 µM Al pre-treatment is shown in Table 1. At 7 dpi, the progress of disease was highest in case of BSO treated seedlings infected with fungal spores.

The mean DSI of these seedlings reached highest value in comparison to control seedlings infected with *Fusarium.* Al pre-treated seedlings infected with fungal spores showed least disease symptom with lowest mean DSI value.

Oxidative burst of ROS was maximum for BSO treated seedlings infected with *Fusarium* (Fig 4). The first burst was apparent after 4 hours post inoculation (hpi). After 48hpi, ROS generation in case of BSO pre-treated seedlings infected with *Fusarium* was twice as compared to Al pre-treated seedlings. The first oxidative burst in case of *Fusarium* infected seedlings was observed after 16hpi. Al pre-treated seedlings were observed to show least generation of ROS during the observation period. Moreover, Al pre-treated seedlings infected with Fusarium showed a decline in ROS accumulation after 16 hpi.

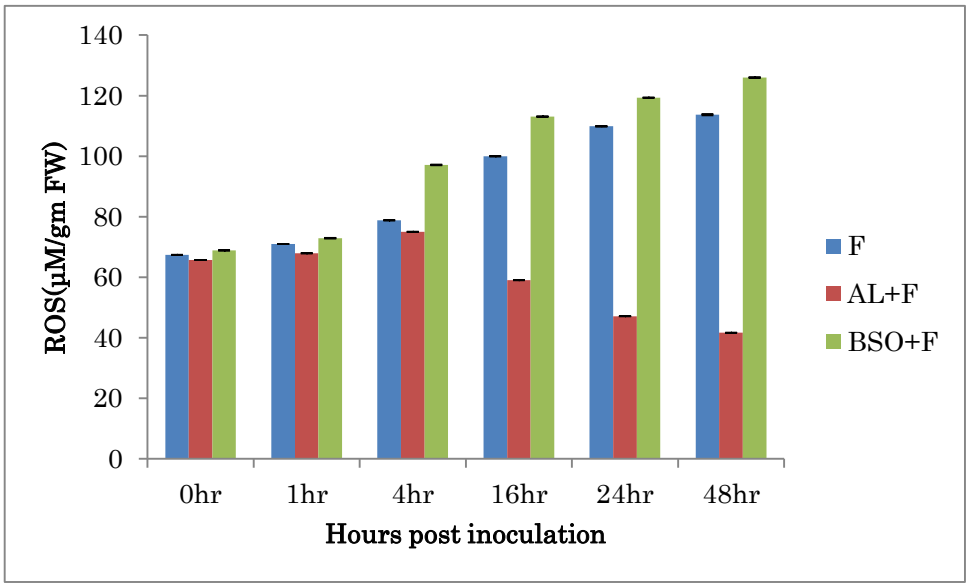


Figure 4. Effect of *Fusarium* inoculation on ROS accumulation in wheat seedlings at different time points. F-shows changes in ROS accumulation in *Fusarium* infected seedlings. Al+F-shows changes in ROS accumulation in Al pre-treated seedlings infected with *Fusarium*. BSO+F-shows changes in ROS accumulation in BSO pre-treated seedlings infected with *Fusarium*. Error bars indicate standard error.

Lactophenol cotton blue staining of leaves revealed fungal mycelial structure inside the tissue in case of infected seedlings. Highest manifestation of fungal mycelia was observed in case of seedlings pre-exposed to BSO followed by fungal infection. However, Al pre-treated seedlings infected with *Fusarium* showed very negligible mycelia structure in leaf tissues when examined under light microscope (Fig 5).

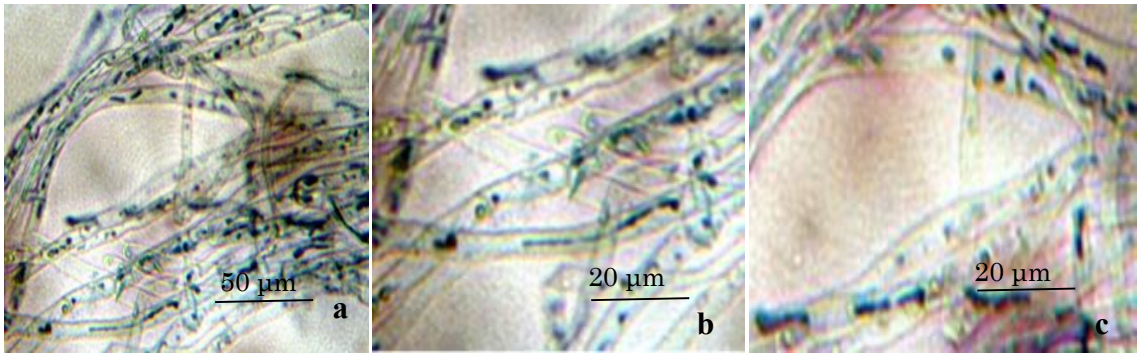


Figure 5. Histology of wheat seedlings in response to *Fusarium oxysporum* at 7dpi. (a) mycelial growth on leaves of BSO pre-treated seedlings infected with *Fusarium*. (b) mycelial growth on leaves of control seedlings infected with *Fusarium*. (c) mycelial presence in leaves of Al pre-treated seedlings infected with *Fusarium*.

DISCUSSION

Glutathione the "master antioxidant" is a ubiquitous non-protein tri-peptide thiol known to serve key functions in protecting cells from oxidative damage [24] Thereby, increasing glutathione synthesis is considered as a way to increase cellular defense against oxidative stress. The pathway of glutathione synthesis, recycling and metabolism are well established. Glutathione synthesis is a two step process involving γ-ECS and glutathione synthetase. Manipulating the enzymes involved in glutathione synthesis is a good approach to enhance tolerance to oxidative stress. In absence of heavy metals the reaction catalysed by γ-ECS is considered as the rate limiting step for glutathione synthesis, because the activity of this enzyme is controlled through feedback inhibition by glutathione and is dependent on availability of cysteine [25]. This view was supported by the observation that the expression of *E coli gshI* gene, encoding γ-ECS in poplars, resulted in increase foliar glutathione levels in poplars [26] Similar expression of γ-ECS in tomato could restore heavy metal tolerance to the *cad2* mutant Arabidopsis to certain extent. Furthermore, the expression of γ-ECS activity is reported to increase by heavy metal application. Increase in the γ-ECS activity turns on the over-expression of glutathione in the system. [27] demonstrated increased level of glutathione biosynthesis in response to cadmium in tolerant pea genotypes. Similar constitutively elevated GSH biosynthesis is linked to nickel tolerance and hyperaccumulation [28]. In the present study we found that the enzyme γ-ECS was up regulated in wheat seedlings with 50 µM concentration of Al pre-treatment. However, BSO treated wheat seedlings showed a decline in the γ-ECS enzyme activity. The up-regulation of γ-ECS in Al pre-treated seedlings induced an increase in the total glutathione abundance in wheat. Thus, it is suggested that γ-ECS is the rate limiting enzyme in the glutathione biosynthetic pathway in wheat plants under our experimental conditions. The rapid and steady increase in glutathione abundance in infected seedlings has also been observed in other plant-pathogen interactions [29]. Changes in glutathione content during incompatible interaction between plants and avirulent pathogens have been reported [9]. Plants with disrupted synthetic pathway

contain lower levels of glutathione and become more susceptible to pathogens [30]. On the other hand, plants exhibiting higher levels of glutathione develop resistance against broad range of pathogens. Application of different concentrations of GSH to suspension cultures of bean cells induced several genes encoding enzymes participating in the biosynthesis of lignin and phytoalexins. GSH supplementation to soybean cells mimicked induction of chalcone synthase, the expression of which occurs as a result of fungal elicitor stimulation. Significant increase in GSH levels occurred as a result of enhance resistance of melon and tomato against *Fusarium oxysporum* brought about by herbicides [7]. Glutathione deficient Arabidopsis mutant *pad2* reveal increased susceptibility to several pathogens like the bacterium *Pseudomonas syringae*, to the oomycete *Phytophtora brassicae* [14] and to the fungus *Alternaria brassicicola* [1]. Further mutants, *cad2* and *rax1* showed susceptibility to avirulent pathogen *P. syringae* concomitant with decline in the transcript levels of genes involved in plant disease resistance [31]. Decreased level of glutathione impaired defense against pathogens by disrupting its role as a redox buffer and in its involvement in the biosynthesis of glucosinolates and phytoalexin camalexin [32]. In the present study BSO treated seedlings showed depletion in the glutathione content along with reduction in the γ-ECS activity. Such seedlings when infected with *Fusarium* were more susceptible to the pathogen as compared to control seedlings infected with *F oxysporum* spores. Pathogens are selective markers in plant evolution because they can severely reduce plant fitness and growth. Literature provides many examples of reduction in yield and plant fitness by pathogens. For example, infection with bacteria *Pseudomonas syringae* to Arabidopsis causes severe reduction in host fitness [32]. *Sclerotinia sclerotiorum* infection to *Mentha arvensis* caused reduction in growth, oil yield and induced biochemical changes [33]. However, in the present study reduction in cellular glutathione pool increased susceptibility of wheat seedlings *Fusarium* attack. On the contrary, Al primed seedlings could alleviate *Fusarium* induced inhibition of root growth. Thereby, over-expressed glutathione is suggestive of playing crucial role in maintaining the health status of the wheat seedlings. Assessment of disease index is the vital step in characterizing the success of any disease management practice. Relative magnitude of success in disease management is decided on the basis of disease assessment. In our study, maximum disease suppression was observed in case of glutathione over-expressing seedlings at 21dpi. Whereas at 21 dpi, BSO treated seedlings when infected with *Fusarium* showed highest disease incidence. These observations correlate over-expressed glutathione with the development of disease resistance in wheat seedlings. Effectiveness of over-expressed glutathione in suppressing the disease progress suggests the importance of glutathione as a key antioxidant.

Oxidative burst is an early response in plant-pathogen interactions. ROS including singlet oxygen ($^1O_2$), superoxide ($O_2^{\cdot}$), hydroxyl radical (·OH) and hydrogen peroxide (H_2O_2) are all by-products of aerobic metabolism and regarded as damaging cytotoxic agents. Accumulation of ROS is known to cause damage to cells by oxidation of proteins, enzymes and nucleic acid. However,

ROS has been established as a powerful signaling component in plant defense system against pathogens [34]. Plants have developed a plethora of ROS scavenging systems including low molecular weight compounds like ascorbate and glutathione and many classes of antioxidative enzymes [35]. In the present study, enhanced level of reduced glutathione in Al pre-treated seedlings minimized ROS activity and protected the seedlings from the oxidative stress imposed by the pathogen. A highly reduced state of glutathione is essential for active protection of proteins. In *Fusarium* infected seedlings although the total glutathione pool was increased, it was unable to protect the seedlings from the oxidative stress. The enhancement of total glutathione pool was due to increase in the oxidized form of glutathione which was unable to protect the seedlings from oxidative damage. Thus, we find glutathione mediated resistance in wheat seedlings against *Fusarium* not only depends on its cellular concentration but also on the oxidation/reduction state of the thiol. ROS accumulated to the threshold level can directly act against phytopathogens or participate in other signaling pathways like reinforcement of the cell wall, programmed cell death, synthesis of secondary metabolites, activation of defense genes and defense hormones [37]. Reinforcement of cell walls are effective barriers that are induced at the site of pathogen attack. Increased abundance of fungal mycelia in leaves of BSO treated wheat seedlings infected with *Fusarium* indicates the inability of the system to resist pathogen entry. The depleted glutathione content in the system was insufficient to keep the ROS value below threshold level through the ROS scavenging machinery. Unlike leaves of Al pre-treated seedlings infected with *Fusarium* showed very little presence of fungal structures inside the leaf tissue. Thereby, over-expressed glutathione is indicative of playing active role against pathogen infection in wheat seedlings by participating in the ROS detoxification machinery. Further, it maintains the cell wall entity and integrity by restricting pathogen entry.

4. CONCLUSION

It is summarized that priming of wheat seedlings with 50 μM concentration of Al induces a rapid and steady accumulation of glutathione. This abundance of glutathione was due to enhanced γ-ECS activity. The enhanced level of reduced glutathione in Al pre-treated seedlings offered resistance against the *Fusarium* pathogen. Unlike Al pre-treated seedlings, *Fusarium* infected seedlings were unable to defend themselves against *F oxysporum* infection due to reduction in the level of reduced glutathione. Inducing resistance in wheat seedlings against pathogen by over-expressing glutathione through Al priming is a novel area in plant-pathogen interaction.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

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HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

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CONFLICT OF INTEREST

No conflict of interest exists between the authors

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